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Prosjektoppgave
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**Intima-media thickness of common carotid and brachial arteries and
prothrombin fragment 1+2 are associated with diastolic dysfunction in
patients with myocardial infarction**

Aims: To investigate the association between intima-media thickness of brachial and common carotid arteries and factors of the coagulation- and fibrinolysis system with diastolic dysfunction in patients with a previous myocardial infarction.

Patients and methods: One hundred and five patients, men (72 %) and women (28%) aged between 32-73 years with a history of previous acute myocardial infarction were included. An age-matched control group with no cardiovascular risk factors was used as a comparison. B-mode ultrasound of common carotid and brachial arteries and echocardiography were evaluated in all subjects. Calculated intima-media area (cIMa) of the common carotid and brachial arteries and tissue Doppler imaging (TDI) were examined. Factors of the coagulation- and fibrinolysis system were also measured.

Results: Prothrombin fragment 1+2 was significantly higher in patients with previous myocardial infarction compared to the control group ($P<0.01$). Early diastolic filling peak velocity ($E'-v$) and late diastolic filling peak velocity ($A'-v$) was significantly lower among patients with previous myocardial infarction than in the control group ($P<0.01$ respectively

P<0.001). Late diastolic filling peak time was significantly and positively associated with log Prothrombin fragment 1+2 (P<0.001), calculated common carotid cIMa (P<0.02), systolic blood pressure (P<0.01) and to IMT of brachial artery (P<0.02) in patients with a previous MI. In stepwise multiple regression analysis, log Prothrombin fragment 1+2 remained the only variable with independent significant correlation to late diastolic filling peak time.

Conclusions: Diastolic dysfunction is correlated to cIMa of common carotid and brachial arteries, systolic blood pressure and Prothrombin fragment 1+2 in patients with myocardial infarction. Thus, atherosclerosis, diastolic dysfunction and coagulopathy are tightly interrelated disorders in patients with myocardial infarction.

Introduction

Tissue Doppler imaging (TDI) is a well-established ultrasound technique. TDI is suitable measuring left ventricle diastolic and systolic function in different pathologies^{1,2}. Left ventricular diastolic function has been shown to be an important predictor of morbidity and mortality after acute myocardial infarction³.

Thrombin is considered having a central role in the pathophysiology of cardiovascular disease⁴. A crucial event in the coagulation cascade is the conversion of prothrombin to thrombin. Prothrombin fragment 1+2 is a polypeptide released from the prothrombin during this transfer. Measurement of circulating levels of prothrombin fragment 1+2 has been regarded as an indicator of thrombin generation in vivo^{5,6}. Previous studies have indicated that prothrombin fragment 1+2 levels are predictors of major coronary events⁷⁻¹⁰. Other studies have failed to show a relationship between prothrombin fragment 1+2 and coronary heart disease^{11,12}.

Intima-media thickness (IMT) of the carotid arteries is an indicator of a subclinical atherosclerosis which, in previous studies, has been shown to predict the incidence of cardiovascular disease¹³⁻¹⁵.

Indications that thrombin generation may have an unfavorable effect on the thickening of the arterial wall in subjects free of clinical cardiovascular disease has been reported¹⁶.

The aim of this study was to examine the relationship between late diastolic filling peak time and factors of the coagulation- and fibrinolysis system, cIMa of the common carotid and brachial arteries in patients with a previous history of myocardial infarction.

METHODS

Subjects

One hundred and five patients, men (72 %) and women (28%) aged between 32-73 years with a history of previous acute myocardial infarction were included. The patients were recruited from the department of Cardiology at Karolinska University Hospital Huddinge, Sweden. A majority of the patients were recruited at the post CCU, 2-3 days after the myocardial infarction. The examinations of this study were performed 1-12 months after the index event. Ninety percent of the patients in this study were examined 3 months after the myocardial infarction. The control group consisted of ten male volunteers aged between 36-71 years.

The exclusion criterion was known diabetes mellitus. Only patients with previously well-known and established diabetes were excluded. All subjects gave informed consent after written and oral information. The ethics committee of the Karolinska Institute at Karolinska University Hospital Huddinge approved the study.

Acute myocardial infarction was defined using the criteria of the European Society of Cardiology and the American College of Cardiology¹⁷. Thus, patients were diagnosed as having an acute myocardial infarction if they had two values of serum troponin T greater than 0.05 g/L together with either typical symptoms or new Q-waves in at least two of the twelve standard electrocardiographic leads, or electrocardiogram changes indicating acute ischemia (ST-elevation, ST-depression, or T-wave inversion).

Measurements

Venous blood was drawn after an overnight fast and 5 min of supine rest for determination of serum levels of cholesterol, triglycerides and fasting plasma glucose using established methods. Venous blood was also drawn with minimal cuff pressure for analysing the hemostatic markers including fibrinogen, von Willebrand factor, Plasminogen activator inhibitor 1 (PAI-1) and prothrombin fragment 1+2. The citrated blood samples were centrifuged within 30 minutes, and plasma was immediately frozen in aliquots and stored at -70°C until analysis. PAI- 1 activity was determined by using the Spectrolyze PAI-1 kit (Biopool AB) on the citrated plasma samples that had been stored at -70°C. Von Willebrand factor antigen was measured by a commercially available ELISA method (Liatest® vWF kit, Stago provided by Triolab AB). Fibrinogen levels in plasma were determined by conventional techniques (Sysmex CA-1500). Level of prothrombin fragment 1+2 was assessed by using enzyme-linked immunosorbent assay kits, Enzygnost F1+2 (Behring).

Resting blood pressure was measured in the right arm after about 10 min supine rest. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²).

Smoking status was assessed by a questionnaire.

Tissue Doppler imaging echocardiography

All studied individuals were examined with echocardiography and TDI using a GE Vingmed System FiVe (Horten, Norway). A standard phased array 2.5 MHz multifrequency transducer was used. All recordings were performed at the end of expiration from apical four chambers (4CH) and two chamber (2CH) view with the subjects in left lateral position. Cine loops of two consecutive heartbeats were acquired in each case with a high temporal resolution (90-147 frames/s, mean 113). The formatted raw data containing both grey scale and TDI information were stored as IQ-data on magneto-optical disk and then transferred to a Macintosh computer for off-line analysis employing the commercially available software Echopac version 6.3.6 (GE Vingmed). The TDI analysis was performed from an optimal measuring position set at the basal segment of each wall (septum, lateral, inferior and anterior wall) of the left ventricle, depending on image characteristics. The true isovolumic contraction period was established in separate experiments by defining off-line in apical five chamber (5CH) view the time point for the closure of the mitral valve and the opening of the aortic valve using anatomical M-mode in 2D grey scale images from two consecutive heartbeats. The true isovolumic relaxation period was defined similarly by aortic closure and mitral valve opening. Anatomical colour M-mode extracted from TDI and the myocardial tissue velocity profile from the basal septum were recorded for the same heartbeats. The grey scale anatomical M-mode images of the mitral and aortic valve and colour anatomical M-mode images and velocity profiles from the basal septum were then organised and synchronised according to ECG signal using Microsoft® Power Point for Windows version 9.0. The following variables in the tissue velocity curve were measured.

Isovolumic contraction time (IVCT): A period of time between the zero crossing point for the ascending limb of the positive isovolumic velocity wave and the zero crossing point for the ascending limb of the myocardial tissue velocity curve at the beginning of the systolic ejection. During this period of time the tissue velocity profile usually shows biphasic pattern of motion, i.e. a positive deflection followed by a negative wave but single-phase movements (positive or negative) may occur as well. In some of these cases, the zero crossing reference points were not available and the beginning and the end of IVCT were defined using the anatomical M-mode images of the closure of the mitral and the opening of the aortic valve.

Isovolemic relaxation time (IVRT): A period of time between the zero crossing point for the descending limb of the systolic myocardial tissue velocity curve at the end of the systolic ejection and the zero crossing point for the one-phase (ascending or descending) or two-phase tissue velocity curve at the start of the diastolic E'-wave. During this period of time the tissue velocity profile usually shows two-phase tissue motion, i.e. a negative deflection followed by a positive wave but single-phase movements (positive or negative) may occur as well. If the zero crossing reference points could not be established, IVRT was defined using the anatomical M-mode images of the aortic closure and the opening of the mitral valve. Only in 3 of 105 investigated patients the zero point was not clearly identified and anatomical M-mode images were used to identify IVRT and IVCT. The myocardial performance index (MPI) was calculated off-line according to the equation $(IVRT + IVCT) / \text{LV ejection time}$.

The intra-observer variability for different systolic and diastolic parameters in the velocity profile varied between 5 and 10%, as described earlier¹⁸.

Echocardiography

All patients underwent a standard echocardiographic evaluation, using a 2.5 MHz transducer (System Five, GE Vingmed, Horten, Norway). The echocardiographic studies were performed

with the subject in left lateral decubitus, position. One physician unaware of the other patient data recorded all the echocardiograms. Left ventricular internal dimension and interventricular septum thickness and ejection fraction were measured according the recommendations of the American Society of Echocardiography¹⁹. The motion of the AV-plane was assessed from the four-chamber apical view, using M-mode as described previously²⁰.

Carotid B-mode ultrasonography

The right and left carotid arteries were examined with a duplex scanner (Aspen, Acuson, Mountain View, Ca, USA) by using a 7 MHz linear array transducer. The same trained sonographer performed all scannings. The far wall of the common carotid artery (CCA), 0,5 to 1,0 cm proximal to the beginning of the carotid bulb, was used for measurements of the IMT and lumen diameter. The examinations were video taped for subsequent analyses by a computer system²¹ with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 10 mm long section of CCA in late diastole, defined by a simultaneous electrocardiographic recording. The mean values of the IMT and lumen diameter within the 10 mm long section were calculated. The differences between repeated measurements of IMT and lumen diameter, by using the automated analysing system, were 3.2 % and 0.6 % (coefficient of variation), respectively (with an IMT of 0.48 to 1.04 mm and a lumen diameter of 4,34 to 7,91 mm). To compensate for the stretching effect of arterial distension (secondary to increased arterial pressure) on the wall thickness, the cross-sectional intima-media area was calculated by using the formula $3.14 [(lumen\ diameter/2 + intima-media\ thickness)^2 - (lumen\ diameter/2)^2]$. This calculated intima-media area (cIMa), but not the IMT, has been shown to be unaffected by variations in artery distension secondary to changes in blood pressure²². The ultrasonographic methods used have been described in detail previously^{23,24}.

Brachial B-mode ultrasonography

The ultrasound procedures for assessing IMT of brachial artery were performed as described in the international guidelines by Corretti et al²⁵. The patients were examined in the morning after fasting since midnight. The patients were told not to use long-acting nitroglycerin or calcium channel blockers drugs 36 hours before the examination. A high-resolution ultrasound scanner (System Five, GE Vingmed, Horten, Norway) with a 10.0-MHz linear array transducer was used. After 10-minute equilibration period at rest in the recumbent position, a single dedicated ultrasonographer performed measurements of the left brachial artery. Scans of the brachial artery were taken proximal to the antecubital fossa and saved on videotape. Images were digitally acquired from the videotape and measured in random order by a single observer blinded to the conditions under which the ultrasonic images were obtained. A computer system²¹ with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 5 mm long section of the brachial artery was used. The far wall of the brachial artery was used for measurements of the IMT and lumen diameter. The cross-sectional intima-media area of the brachial artery was calculated using the same formula as for the common carotid intima-media area.

The differences between repeated measurements of IMT and lumen diameter by using the automated analysing system, were 3.0 % and 1.4 % (coefficient of variation), respectively.

Statistical analysis

Results are reported as mean \pm SD except where indicated otherwise. All data analyses were done using Statistica for Windows software version 7.0. Mann-Whitney U test, X² test, Spearman's correlation of coefficient and stepwise regression analysis were performed. Since coagulations factors were not normally distributed a log transformation were performed. Statistical significance was taken at level of $P < 0.05$.

Results

Baseline characteristics of the study are shown in Table 1. Total cholesterol level was significantly higher in the control group than in the study group ($P<0.01$). Prothrombin fragment 1+2 and PAI-1 was significantly higher in patients with previous myocardial infarction compared to the control group ($P<0.001$ respectively $P<0.01$). Regarding smoking habits there were more former smokers and less snuff users in the study group ($P<0.05$).

Table 2 shows TVI-data between the two groups. Early diastolic filling velocity ($E'-v$) and late diastolic filling peak velocity ($A'-v$) was significantly lower among patients with previous myocardial infarction than in the control group ($P<0.01$ respectively $P<0.001$). There were no significant differences between the two groups regarding; left ventricle ejection time (S2), the mean of isovolumic contraction time (IVCT), the mean of isovolumic relaxation time (IVRT) or myocardial performance index (MPI).

Table 3 demonstrates the correlation between late diastolic filling peak time and study variables in the two groups. In patients with previous myocardial infarction, late diastolic filling peak time was significantly and positively associated with log Prothrombin fragment 1+2 ($P<0.001$) and with cIMa of the common carotid and brachial arteries ($P<0.05$) also shown in the figures 1 and 2 respectively. Moreover late diastolic filling peak time correlated positively and significantly to systolic blood pressure ($P<0.01$) and to IMT of brachial artery ($P<0.02$). In the control group, late diastolic filling peak time was significantly and positively associated with heart rate ($r=0.66$) ($P<0.04$).

When cIMa of the common carotid and brachial arteries, systolic blood pressure and log Prothrombin fragment 1+2 were entered into a stepwise multiple regression analysis, log

Prothrombin fragment 1+2 remained the only variable with independent significant correlation to late diastolic filling peak time, in patients with previous myocardial infarction.

At the time of inclusion 104 (99%) of patients with previous myocardial infarction were taking Aspirin, 97 (92%) B-blockers. Statins were given to 97 (92%) patients and 26 (25%) received Angiotensin converting enzyme inhibitor.

Discussion

The main results of this study are that late diastolic filling peak time is significantly correlated to systolic blood pressure, to the level of prothrombin fragment 1+2, to IMT of brachial artery and to the cIMA of carotid artery, in patients with a previous history of myocardial infarction. Thus, atherosclerosis, diastolic dysfunction and coagulopathy are tightly interrelated disorders in patients with myocardial infarction.

We rose the question what an elevated level of prothrombin fragment 1+2 represents. Its half-time is approximately 90 minutes, which could make it a marker of ongoing coagulation.

Several studies have found a correlation between elevated plasma levels of prothrombin fragment 1+2 and presence and severity of atherosclerosis^{26,27}. However, other studies did not find any association between prothrombin fragment 1+2 and the severity of angiographically measured⁸ atherosclerosis, or presence of coronary heart disease^{11,12}.

Lopez et al found a significant elevation in prothrombin fragment 1+2 among elderly, patients with acute myocardial infarction, hematologic malignancies and pregnancy. They interpreted this as a sign for marked clotting activity²⁸. A review by Fareed stated that the hypercoagulable state shown in pregnancy, during which prothrombin fragment 1+2 is elevated, may increase the risk for venous thrombosis. However concerning atherosclerosis,

they claimed that atherosclerosis is associated with vascular dysfunction and may require other markers than Prothrombin fragment 1+2. On the other hand, their review suggests that prothrombin fragment 1+2 may be used to supervise the treatment of patients with previous myocardial infarction²⁹.

In line with our findings in the present study, Gyöngyösi et al concluded that elevated levels of prothrombin fragment 1+2 were related to signs of plaques instability in patients with angina and non-ST elevation myocardial infarction. Moreover they declared that biomarkers of plasmin activation system might function as non-invasive determinants in high risk populations³⁰.

Páramo et al demonstrated that prothrombin fragment 1+2 is correlated to IMT in subjects without clinically explicit atherosclerotic disease. In addition they proposed that by reducing the prothrombin fragment 1+2 level this would tentatively reduce the development of atherosclerotic disease¹⁶. However, in our study we eliminated the effect of IMT among other variables in a multistep analysis, resulting in that only the level of prothrombin fragment 1+2 remained significantly associated with late diastolic filling peak time. To our knowledge there has not been any other study examining the correlation between late diastolic filling peak time and prothrombin fragment 1+2. We found no associations between late diastolic filling peak time and the other measured factors from the coagulation and fibrinolysis system.

Our study revealed a significant positive relationship between late diastolic filling peak time and IMT of brachial artery as well as between late diastolic filling peak time and cIMA of carotid artery. This could demonstrate a positive correlation between two atherosclerotic markers and diastolic dysfunction. In line with our study Gonzales et al demonstrated that patients with ischemic heart disease and patients with carotid stenosis and stroke had higher late diastolic peak velocity as well as higher (A/E)-ratio, early diastolic time, early diastolic

acceleration and early diastolic deceleration. Both groups were concluded having diminished left ventricle compliance compared to a healthy control group. Their study showed that patients with ischemic heart disease had more prominent diastolic dysfunction than patients with previous stroke and carotid stenosis³⁰.

Thune and Solomon³¹ discuss left ventricular diastolic function after myocardial infarction. After an episode of myocardial infarction both the active relaxation and the passive filling during diastole are affected. The active relaxation is impaired by diminished recoiled phenomena among other factors. Moreover left ventricular stiffness is altered depending on the degree of the infarction and the remodeling after. The ventricular stiffness is boosted by the interstitial edema and fibrosis but dilatation of the heart works against this effect. Another cause of diastolic dysfunction is electromechanical dyssynchrony following myocardial infarction which may result in segments contracting even in the early filling phase³¹.

Eventually preexisting conditions such as diabetes and hypertension worsen the prognosis after the infarction^{31,32}. Additionally Jogestrand et al's study demonstrated, that there are only, if any, a weak correlation between common carotid IMT or cIMA and the artery stiffness of the common carotid artery wall³³. Another study revealed that more prominent atherosclerosis was required to identify atherosclerotic plaques by determining wall elasticity³⁴. The outcomes in these studies are in line with our findings. We observed that diastolic dysfunction, diagnosed by TDI, correlates to a hypercoagulable state and subclinical atherosclerosis in the arteries, in patients with previous history of myocardial infarction.

Moreover in this study we displayed that late diastolic filling peak time was positively related to systolic blood pressure. In hypertensive patients Tanaka et al demonstrated that Losartan (Angiotensin II type 1 receptor blockade) treatment improved diastolic function confirmed by TDI. The study revealed that the ratio of early to late diastolic filling for the transmitral flow velocity increased after Losartan treatment as the peak systolic and early diastolic myocardial

velocities and myocardial velocity gradients in the ventricular septum and left ventricle posterior wall did. On the other hand, Zakynthinos et al illustrated that Losartan treatment did not affect the diastolic function of the left ventricle (early diastolic filling velocity [E wave], late diastolic filling velocity [A wave], ratio of E/A waves, isovolumic relaxation time), which were abnormal at baseline. Nevertheless the medication reduced left ventricular hypertrophy proportionally to blood pressure³⁵. In our study 25% of the study group were on angiotensin converting enzyme inhibitor, this could have influenced the outcome. It is reasonable to believe that patients in our study taking this treatment could have shown a stronger correlation between systolic blood pressure and late diastolic filling peak time, without the medication. Further studies need to explore the correlation between late diastolic filling peak time and systolic blood pressure.

We need to consider some methodological aspects of our study. The study group includes patients with previous episodes of myocardial infarction. The disadvantage of such a population is that almost all participants will be given medical treatment. Kienast et al illustrate that oral anticoagulants was associated with reduced mean level of prothrombin fragment 1+2 in patients with angina pectoris⁸. Patients receiving anticoagulant therapy were therefore excluded in our study not to influence the outcome.

Subjects in the control group had higher levels of cholesterol than the study group. Almost all of the patients with previous history of myocardial infarction received statins, which the subjects in the control group did not. This could explain the above mentioned finding. A correlation that might have been hidden in the study group is the association seen in the control group between late diastolic filling peak time and heart rate. The lack of such a relation in the study group might be due to the high prevalence of beta-receptor blockage treatment (92%) in patients with previous MI. A limitation with this study is the small number of subjects in the control group. Nevertheless the two groups were of comparable age.

Prospective studies could increase our knowledge of the relationship between diastolic dysfunction, a hypercoagulable state, and subclinical atherosclerosis and elevated systolic blood pressure in patients with previous history of myocardial infarction.

In conclusion, we observed a tight correlation between atherosclerosis, diastolic dysfunction and coagulopathy in patients with myocardial infarction.

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Table 1. Baseline characteristics of the study subjects.

	Patients with previous MI N = 105	Controller N = 10
Age (years)	58 ± 9	54 ± 12
Systolic blood pressure (mmHg)	137 ± 20	129 ± 15
Diastolic blood pressure (mmHg)	80 ± 10	78 ± 5
Heart rate (bpm)	58 ± 10	61 ± 13
Total cholesterol (mmol/L)	4.7 ± 0.8**	5.5 ± 0.8
Triglycerides (mmol/L)	1.7 ± 1.1	1.1 ± 0.3
Fasting plasma glucose (mmol/l)	5.4 ± 1.4	5.2 ± 0.4
BMI (kg/m ²)	27 ± 4.0	26 ± 3
Ejection fraction (%)	53 ± 9.0	52 ± 5
Prothrombin fragment 1+2 (nmol/l)	0.9 ± 0.8***	0.5 ± 0.2
von Willebrand factor (IU/ml)	1.32 ± 0.43	1.2 ± 0.37
Plasminogen activator inhibitor 1 activity (IU/ml)	20.2 ± 18.2**	7.58 ± 6.63
Fibrinogen (g/l)	3.3 ± 0.7	3.10 ± 0.65
Hs-CRP, mg/l	3.1 ± 4.90	1.50 ± 1.78
Calculated brachial intima media area (mm ²)	5.0 ± 1.5	5.0 ± 2.0
Calculated carotid intima-media area, mean (mm ²)	18 ± 5	15 ± 4
Smoking habits, n (%)		
Current smoker	20 (19)	2 (20)
Never smoked	19 (18)	4 (40)
Prior smoker	62 (59) †	2 (20)
Snuff user	4 (4) †	2 (20)

*P<0.05, ** P< 0.01, *** P< 0.001 Mann Whitney test, † P<0.05, †† P< 0.01 X² test.

Table 2- Tissue Doppler imaging (TDI) data of patients with cardiovascular disease (CVD) and control group.

TDI data	CVD group N = 105	Control group N = 10
S2 (ms)	298 ± 30	
E'-v (cm/s)	-5.8 ± 2.0**	
A'-v (cm/s)	-6.3 ± 2.0***	
E'/A'	1.0 ± 0.4	
E' -T (ms)	189 ± 35	
A' -T (ms)	120 ± 18	
IVRT (ms)	96 ± 24	
IVCT mean	71 ± 18	
MPI	0.6 ± 0.1	

*P<0.05, ** P< 0.01, *** P< 0.001 Mann Whitney test. S systole, V indicates velocity, E'-v early diastolic filling peak velocity,

A'-v late diastolic filling peak velocity, E'/A' ratio of the early to late peak diastolic velocity. E' -T early diastolic filling time,

A' -T late diastolic filling time. IVRT isovolemic relaxation time. IVCT isovolemic contraction time. MPI myocardial performance index.

Table -3 Correlation coefficients between late diastolic filling time and variables in focus.

	CVD group (n=105)	
	R	P-value
Age (years)	-0.02	0.88
Systolic blood pressure (mmHg)	0.26	0.01
Diastolic blood pressure (mmHg)	0.00	1.0
Heart rate (bpm)	-0.14	0.16
Total cholesterol (mmol/l)	0.03	0.80
Triglycerides (mmol/l)	-0.06	0.58
Fasting plasma glucose (mmol/l)	-0.10	0.31
BMI (kg/m ²)	0.03	0.78
Ejection fraction (%) (Simpson)	-0.08	0.47
Log Prothrombin Fragment 1+2 (nmol/l)	0.37	0.001
von Willebrand factor (IU/ml)	-0.14	0.18
Plasminogen activator inhibitor 1 activity (IU/ml)	-0.08	0.43
Fibrinogen (g/l)	-0.17	0.10
Hs-CRP, mg/l	-0.07	0.50
Calculated common carotid intima media area, mm ² (mean of right and left side)	0.24	0.02
Calculated brachial artery intima media area (mm ²)	0.23	0.02

CVD, cardiovascular disease, CRP, High sensitivity C-reactive protein

Figure-1

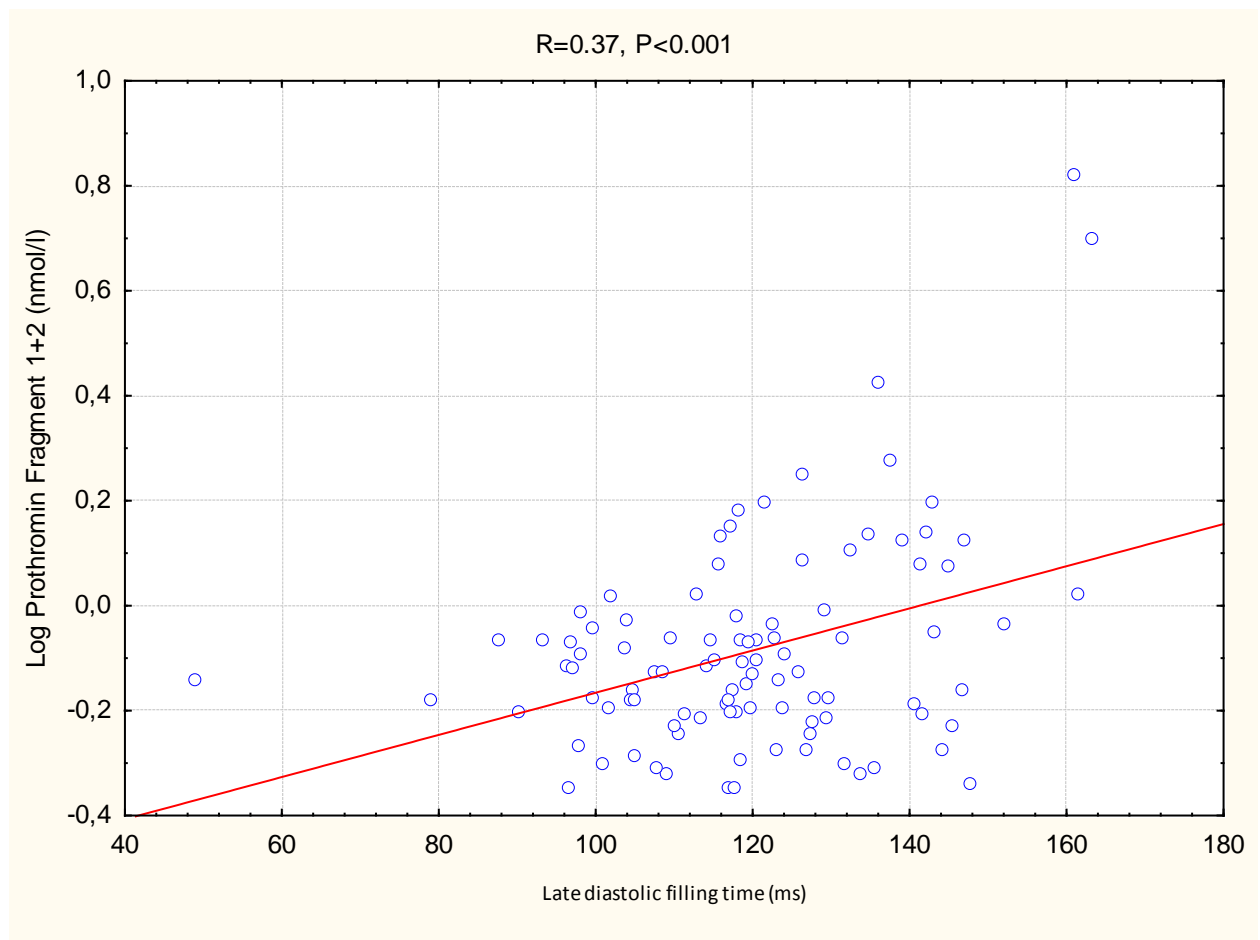


Figure-2

